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REVIEW

The role of polymorphonuclear neutrophils during HIV-1 infection

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Abstract It is well-recognized that human immunodeficiency virus type-1 (HIV-1) mainly targets $CD4^+$ T cells and macrophages. Nonetheless, during the past three decades, a huge number of studies have reported that HIV-1 can directly or indirectly target other cellular components of the immune system including $CD8^+$ T cells, B cells, dendritic cells, natural killer cells, and polymorphonuclear neutrophils (PMNs), among others. PMNs are the most abundant leukocytes in the human circulation, and are known to play principal roles in the elimination of invading pathogens, regulating different immune responses, healing of injured tissues, and maintaining mucosal homeostasis. Until recently, little was known about the impact of HIV-1 infection on PMNs as well as the impact of PMNs on HIV-1 disease progression. This is because early studies focused on neutropenia and recurrent microbial infections, particularly, during advanced disease. However, recent studies have extended the investigation area to cover new aspects of the interactions between HIV-1 and PMNs. This review aims to summarize these advances and address the impact of HIV-1 infection on PMNs as well as the impact of PMNs on HIV-1 disease progression to better understand the pathophysiology of HIV-1 infection.

Introduction

Although more than three decades have passed since the discovery of human immunodeficiency virus (HIV)-1, the etiologic agent of acquired immunodeficiency syndrome (AIDS), HIV-1 infection is still an incurable disease. This is, at least in part, due to the fact that HIV-1 almost targets/negatively affects all the cell types of the immune system in infected individuals [1–7]. For instance, $CD4^+$ T cells are the major target cells for HIV-1 infection and replication. Nonetheless, not all $CD4^+$ T cells are preferred for HIV-1 replication, since it replicates very efficiently in activated but not resting $CD4^+$ T cells [8]. The very rapid viral replication in activated $CD4^+$ T cells ensures high viral load as well as the high mutation rate that enables to HIV-1 escape both immune responses and antiviral therapeutics. On the other hand, infection of resting $CD4^+$ T cells enables HIV-1 to become latent(transcriptionally silent), and thus unrecognizable to the immune system and antiviral therapeutics, ensuring viral persistence in HIV-1 patients [9]. Macrophages are the second most favored cells for HIV-1 infection and replication [10, 11]. However, unlike $CD4^+$ T cells, which are susceptible to HIV-1-related cytopathic effects that result in a massive depletion of $CD4^+$ T cells during the course of HIV-1 infection, macrophages show much more resistance against these cytopathic effects [12, 13]. Interestingly, HIV-1 hijacks this property to ensure its persistence through establishment of a stable latent infection in these cells [13–15]. Moreover, HIV-1 can harness monocytes/macrophages as vehicles to spread throughout the body compartments (such

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as the central nervous system and gut) and between target cells, thereby supporting its persistence [16–18]. HIV-1 can also infect other immune cells such as dendritic cells albeit at a rate of 1 to 2 orders of magnitude lower than infection in $CD4^+$ T cells [19, 20]. Despite the fact that dendritic cells are not considered major targets for direct HIV-1 infection, dendritic cells can enhance HIV-1 infectivity towards $CD4^+$ T cells [16, 17]. In addition, during the course of HIV-1 infection, dendritic cell numbers, phenotypes, and functions are grossly altered resulting in various immunological alterations, (reviewed in [21]). These alterations subsequently inhibit potent anti-HIV-1 immune responses, all of which are required to support HIV-1 persistence. This is also the case with other immune cells such as natural killer cells and basophils/mast cells (reviewed in [21]). Hence, we can say that HIV-1 is able to hijack and exploit almost all cell types of the immune system for its advantage, taking into consideration the individual differences between these cells. Accordingly, it is not surprising to realise that polymorphonuclear neutrophils (PMNs) undergo similar peturbation during the course of HIV-1 infection.

This review aims to address critical issues related to PMNs and HIV-1 infection. However, to begin we will concisely introduce the reader to some of the major biological aspects of PMNs that include: (i) PMNs infiltration, pathogen recognition and elimination; (ii) PMNs cross-talking with other immune cells; and (iii) PMNs and mucosal homeostasis, especially in the gut compartment. This introduction will allow a better estimatation and understanding of the very critical impact of PMNs on health and disease (i.e. during HIV-1 infection). Next we will address the impact of HIV-1 infection on PMN number and function, and then we will address the consequences of PMN alterations on the pathogenesis of HIV-1 infection. Finally, this review will address the role of PMNs in the pathology of gut mucosa and microbial translocation during HIV-1 infection, to improve our understanding of the pathophysiology of HIV-1 infection.

PMNs: roles in pathogen elimination and immune mediation

PMNs are the most abundant leukocytes in the human circulation, constituting up to 60-70% of the total number of circulating leukocytes. These granulocytes are generated in the bone marrow during the process of hematopoiesis at a rate of ~ 100 billion cells per day under normal conditions; this number may reach ~ 1 trillion during serious infections. Relatively speaking, PMNs are short lived cells; however, recent studies have indicated that PMNs may have 10 times (5.4 days) longer lifespans than that previously reported under homeostatic conditions [22]. As professional innate immune cells equipped with several defense mechanisms, PMNs can mediate different effector functions against extracellular pathogens. Intriguingly, it has been recently revealed that PMNs have the capability to eliminate intracellular pathogens including viral pathogens such as HIV-1 [23].

PMNs are characterized by their ability to rapidly infiltrate to the site of infection/inflammation to mediate their effector functions [24, 25]. To accomplish this task, PMNs express a variety of receptors which includes those required for adhesion to endothelial cells during the infiltration process such as selectins, selectin-ligands and integrins, among others [25]. After leaving the vascular compartment, chemo-attractant receptors (such as chemokine and cytokine receptors) facilitate their migration to the site of pathogenic stimulation [26, 27]. In order to detect pathogens, PMNs express several classes of receptors such as toll-like receptors (TLRs), nod-like receptors (NLRs), dectin-1 [28–30], Fc-receptors (FcR) that recognize antibody-opsonized pathogens, and complement receptors that recognize complement-opsonized targets [31–36]. In addition, PMNs express receptors for granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) that participate in enhancing their responsiveness and a metabolic burst, while prolonging their survival once at the site of stimuli [31, 36]. In turn, this helps further recruitment of immune cells to the site of inflammation to effectively eliminate invading pathogens and terminate inflammation, indicating that PMNs can be seen as one of the first lines of defense against invading pathogens.

There are several different mechanisms by which PMNs can eliminate pathogens. These include: phagocytosis, neutrophil extracellular traps (NETs) formation, antibody-dependent cellular cytotoxicity, degranulation and the release of antimicrobial peptides [24, 25, 31, 37, 38]. For instance, once a pathogen is encountered by a PMN, particularly in the circulation, phagocytosis takes place. The presence of serum favors triggering of phagocytosis while inhibiting the induction of NETs [39]. This information indicates that the extracellular milieu may significantly affect the mechanisms of killing employed by PMNs. It is also of considerable importance to realise that PMNs are extremely potent and very efficient phagocytes that can internalize IgG-opsonized particles within less than 60 seconds when compared to other professional phagocytes such as macrophages, which require several minutes to digest similar amounts and types of ingested particles [40–42]. The degradation process takes place once a pathogen or a microorganism is inside the phagosome of a PMN. This is accomplished by mediating the fusion of the PMNs' granules with the phagosome. These granules contain several digesting and hydrolyzing enzymes that act as weapons to destroy the phagocytized pathogens [31]. PMNs further

recruit nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (NOX) to the phagosome to optimally destruct their contents [43, 44].

In another example, PMNs have suicidal capabilities, i.e. to capture and kill invading microorganisms in order to limit their spread. PMNs release highly sticky net-like structures upon infiltration to the site of invasion [45, 46]. These structures have been designated as NETs and are composed of genomic DNA, histones, and various antimicrobials such as calprotectin, α-defensin, and myeloperooidase (MPO) among others, which combine to efficiently eliminate invading pathogens and prohibit their dissemination [45, 46]. Intriguingly, intact PMNs can also release NETs, indicating that NET formation is not only associated with PMNs cell death [47]. Furthermore, PMNs can mediate the killing of infected cells in an antibody-dependent manner via engagement of FcγR with the Fc portion on IgG-opsonized infected cells [48]. Taken together, these data briefly clarify how PMNs can infiltrate, recognize and eliminate pathogens and also highlight their role as key effector cells in the innate immune system.

Indeed, PMNs function is not only restricted to their ability to eliminate pathogens; they can also engage with several types of immune cells to orchestrate immune responses. For instance, *in vitro* and *in vivo* studies have shown that the direct interaction of lipopolysaccharides (LPS)-stimulated PMNs with dendritic cells induces their activation (maturation) and production of tumor necrosis factor alpha (TNF-α) and interleukin-12 (IL-12) [48]. Other studies have also revealed that PMNs are involved in the induction of dendritic cell activation upon the direct interaction of PMNs' surface molecules, such as macrophage antigen-1 (MAC1) and carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1), with corresponding molecules on dendritic cells, namely dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) [49, 50]. Moreover, PMNs have been demonstrated to promote dendritic cell survival through a manner dependent on cell-to-cell contact [51]. Alternatively, activated PMNs release different molecules that can mediate dendritic cell activation in a manner independent on direct contact; these include α-defensins, cathelicidins, lactoferrin, and high-mobility group proteins [52]. Accordingly, these activated dendritic cells can then mediate T cell proliferation and shape their polarization towards distinct helper T cell phenotypes [50, 53], thereby shaping the adaptive immune response. However, under certain circumstances, PMN interaction with dendritic cells may not result in their activation. For example, PMNs-released elastases and ectosomes interfere with dendritic cell activation, in part, through increasing the production of transforming growth factor-β1 (TGF-β1), an immunosuppressive cytokine [54, 55]. These data indicate that PMNs are involved in both immune activation and suppression, which seems to be dependent on the particular microenvironment where interactions take place.

In another example, Silva has shown that PMNs and monocytes/macrophages work together in harmony to mediate effective downstream innate immune responses against both extracellular and intracellular pathogens [56]. Activated PMNs recruit monocytes/macrophages to the site of inflammation through secretion of different attractant molecules such as macrophage inflammatory protein-1α (MIP-1α) and MIP-1β, among others [57–59]. PMNs can then activate recruited monocytes/macrophages and mediate their polarization toward anti-inflammatory or pro-inflammatory subsets according to the microenvironment of the interaction [60]. In turn, these activated macrophages release G-CSF and GM-CSF that prolong the survival of PMNs [61, 62], maximising the PMNs' effector functions. Furthermore, these activated macrophages can then mediate and shape the adaptive immune responses, since macrophages are well-recognized to act as professional antigen presenting cells. Interestingly, recent studies have also shown that NET formation by PMNs is regulated by macrophages in a time- and phenotype-dependent manner (for more details see ref. [63]), which reflects the vital relationship between these types of cells, and also highlights the functional complementarily between these cells [56].

In terms of PMN cross-talking with other types of innate immune cells, such as natural killer cells, Spörri and colleagues have shown that mice PMNs are critical activators of natural killer cells [64]. They have shown that IL-18-derived from PMNs in combination with IL-12, a dendritic cell-derived cytokine, are critical for triggering the secretion of interferon-γ (IFN-γ) from natural killer cells in *Legionella pneumophila*-infected mice. Interestingly, the lack of IFN-γ, as a result of neutropenia in infected mice, has been implicated in their inability to clear the bacterial infection [64]. In line with these data, a later study revealed that natural killer cells from neutropinic mice exhibit hyperproliferation, poor survival, and hyporesponsiveness due to a block in their maturation process at an immature stage [65]. The critical impact of PMNs on natural killer cell functions has also been confirmed in neutropinic-related disorders such as autoimmune neutropenia and severe congenital neutropenia [65]. However, once the natural killer cells are activated by PMNs, they can activate dendritic cells by releasing IFN-γ and TNF-α, or through a contact-dependent activation manner [66]. In turn, these activated dendritic cells can then activate adaptive immune responses, as previously discussed. Furthermore, activated natural killer cells have been observed to promote the activation and survival of PMNs in culture studies, both of which rely on direct cell-to-cell contact and cytokine-dependent mechanisms [67]. These data briefly reflect the very critical relationship between these two types of cells. For more details, Costantini and

Cassatella have comprehensively reviewed the defensive alliance between PMNs and natural killer cells [68].

The impact of HIV-1 infection on PMN count and function

Numeric- and phenotypic-alterations as well as functional defects in PMNs are frequently observed during the course of HIV-1 infection [69–72].

PMNs depletion (neutropenia)

In terms of numerical-alteration, some studies have reported that about 17% of HIV-1 patients exhibit neutropenia [73, 74], while others have reported that up to 50% of HIV-1 patients exhibit neutropenia [75], indicating that neutropenia is relatively common in HIV-1 patients. Recent studies have also indicated that the incidence of severe neutropenia is high in HIV-1 patients living in West Africa, even in those treated with antiretroviral therapy (ART) [76]. Of note ethnic neutropenia is prevalent in individuals of African ancestry [77, 78], which is, at least in part, related to genetic factors [78]. Of particular importance, longitudinal analysis has found that HIV-1 disease progression is directly associated with the severity of neutropenia [75]. Of note, neutropenia has not been only implicated in the disease progression of HIV-1 infection but is also considered as a possible risk factor for HIV-1 transmission during the perinatal period, since a higher PMN count in HIV-1 positive women has been demonstrated to be inversely associated with perinatal HIV-1 transmission risk [79]. PMNs were also shown to play a role in protection against sexual HIV-1 acquisition in adults, as demonstrated by a Taiwanese cohort (which studied HIV-1-exposed but uninfected individuals) [80]. These data reflect the possible impact of neutropenia on disease progression in HIV-1 infected individuals and also the transmission risk to uninfected individuals. Therefore additional investigations are required to further establish the role of neutropenia on HIV-1 disease progression and transmission. However, in this paper we will only address the role of PMNs during HIV-1 infection. Several possible mechanisms, by which HIV-1 infection can contribute to the neutropenia, are mentioned as follows:

- Direct cytopathic effects related to direct HIV-1 infection. Some early studies indicated that HIV-1 could directly infect PMNs, due to the detection of HIV-1 DNA in these cells [81]. This was further supported by the findings of Biswas et al. who showed that 7.8% of HIV-1 patients and 12% of healthy individuals express the CD4 molecule (the primary receptor for HIV-1 entry into target cells) on 39-97% of their PMN populations [82]. In addition, PMNs constitutively express C-X-C chemokine receptor type 4 (CXCR4 or X4), a major co-receptor involved in HIV-1 entry [82]. Even though this suggests that PMN depletion during the course of HIV-1 infection could, in part, be due to direct HIV-1-related cytopathic effects on PMNs in some patients, there is still no clear indication that HIV-1 can directly infect PMNs. Nonetheless, as professional phagocytes, PMNs can internalize HIV-1 by phagocytosis. HIV-1 might also be able to escape destruction by endosomal compartments withini PMNs, as it does in macrophages [83]. Furthermore, HIV-1 can also its Nef protein to inhibit the formation of phagosomes in macrophages by altering endosomal compartment membrane recycling [84]; thus, it could be assumed that HIV-1 could use the same strategy in PMNs to establish a non-canonical (indirect) mechanisms of infection. These hypotheses remain assumptions and cannot fully explain PMN depletion during HIV-1 infection. Therefore, it is of particular importance to highlight that studying the capability of HIV-1 to infect and mediate cytopathic effects in PMNs remain important questions that need to be answered in the near future. However, other explanations do exist to explain neutropenia during the course of HIV-1 infection.
- PMNs apoptosis (bystander apoptosis). Early *ex vivo* studies have demonstrated that PMNs from AIDS patients exhibit remarkable increased rates of apoptosis; however, *in vitro* incubation of PMNs from AIDS patients with G-CSF significantly decreased the rate of apoptosis [85], suggesting a potential benefit of G-CSF in this situation. Other studies have assessed programmed PMN cell-death at different HIV-1 disease stages using TUNEL assays and propidium iodide, and have shown that accelerated PMN apoptosis occurs at different clinical stages, with a remarkable increase in advanced disease stages [86]. Importantly, Fas-mediated apoptosis in PMNs from HIV-1 patients was proposed to be a mechanism that contributes to neutropenia during HIV-1 infection [87]. It is noteworthy that apoptosis in PMNs from HIV-1 patients was shown to be closely associated with the levels of Fas-FasL surface molecules expressed, which are directly associated with viral load [87], indicating that HIV-1 indirectly mediates PMN apoptosis. Other studies have demonstrated that oxidative stress secondary to HIV-1 infection is associated with increased spontaneous PMN apoptosis during the course of HIV-1 infection, since the inhibition of reactive oxygen species (ROS) resulted in decreased PMN apoptosis [88]. Furthermore, the inhibition of ROS decreased caspase-3 hydrolysis, connecting oxidative stress with the intrinsic (caspase-3), but not the extrinsic (caspase-8), apoptotic pathway in mediating PMN apoptosis during HIV-1 infection [88]. Studies in

non-human primates (*Rhesus macaques*) infected with a pathogenic simian immunodeficiency virus (SIV) strain have also demonstrated that PMNs undergo apoptosis [89]. Intriguingly, SIV-infected *Rhesus macaques* with increased PMN apoptosis rates were shown to be associated with faster disease progression [89]. PMN apoptosis in SIV-infected *Rhesus macaques* was also demonstrated to be associated with PMN activation state and ROS production [89]. These data emphasize that the remarkably increased rate of PMN apoptosis during the course of HIV-1 infection is likely, at least in part, to be a possible explanation for their observed depletion during HIV-1 infection [88, 90, 91]; however, other factors may also contribute to neutropenia in HIV-1 patients.

- Affecting the hematopoiesis process. HIV-1 may directly decrease PMNs counts through affecting the hematopoiesis process in the bone marrow. This is thought possible because several studies have demonstrated that HIV-1 can infect certain $CD34^+$ hematopoietic stem cells that express CD4, CCR5 and CXCR4 on their surfaces [92–99]. Moreover, different viral proteins can also directly affect the hematopoiesis process. For example, *in vitro* studies have revealed that the HIV-1 envelope glycoprotein 120 (gp120) can suppress the growth of $CD34^+$ hematopoietic stem cells by inducing endogenous TGF-β, which is a growth inhibitory cytokine [100]. Other studies demonstrated that HIV-1 gp120 can also induce apoptosis in $CD34^+$ hematopoietic stem cells in a Fas-manner dependent [101]. In another example, Nef and Tat viral proteins can suppress the growth of granulomonocytic and myeloid progenitor cells, thereby contributing to the neutropenia observed in HIV-1 infection [102–104]. Deductively, these direct suppressive effects of HIV-1 and HIV-1 proteins on the hematopoiesis process are consistent with the pan-leukocytopenia and other cytopenias such as anemia and thrombocytopenia observed in HIV-1 patients [105]. Interestingly, HIV-1 can also indirectly affect the hematopoiesis process by altering the bone marrow microenvironment (through modulation of cytokines and growth factors) [106–108]. This, in part, can be attributed to the ability of HIV-1 to infect different types of bone marrow stromal cells (e.g. monocytes/macrophages and megakaryocytes) [109–111], which are the source of cytokines, growth factors, and other regulators involved in hematopoiesis.

On the other hand, secondary and opportunistic infections (bacterial, fungal, protozoal, or viral infections) are frequently reported in HIV-1 patients, particularly in advanced disease stages [70, 112–115]. Certain infections may directly contribute to the neutropenia in HIV-1-infected individuals by targeting hematopoietic stem cells, myeloid progenitors, and bone marrow stromal cells, all of which would impair the normal hematopoiesis process. Alternatively, these secondary infections may directly target mature PMNs in the blood circulation. For secondary bacterial infections, HIV-1 patients exhibit an increased risk of *Salmonella* infection [114–116], which is known to cause neutropenia by infecting and suppressing the development of bone marrow hematopoietic stem cells [117]. Similarly, mycobacterial infections such as infection with *Mycobacterium tuberculosis* were also shown to negatively affect the bone marrow and hematopoiesis [118, 119], taking into consideration that *Mycobacterium tuberculosis* infection is relatively prevalent in HIV-1 patients [119, 120]. Cytomegalovirus (CMV) infection is another example of a frequently reported secondary viral infection in HIV-1 patients [121]. Of considerable importance, CMV infection is known to target both bone marrow stromal cells and hematopoietic stem cells, which, in turn, suppress the normal hematopoiesis process resulting in cytopenia, including neutropenia [122]. In addition, studies have shown that HIV-1 patients infected with opportunistic fungal pathogens such as *Pneumocystis carinii*, *Candida albicans*, or *Cryptococcus neoformans* show suppressed myelopoiesis and injured bone marrow [123, 124], thereby affecting the generation of new PMNs at the level of hematopoiesis in the bone marrow, also resulting in neutropenia.

- Increased PMN infiltration rate. The continuous infiltration of PMNs into inflamed lymphatic tissues (e.g. mucosa-associated lymphatic tissues and lymph nodes) and other non-lymphatic tissues that harbor HIV-1 may, in part, provide another explanation for the depletion of PMNs from the circulation of HIV-1 patients (discussed later) [125], especially because PMNs are among the first cells to infiltrate to the site of an immune stimulus, as previously discussed. This assumption, in part, arose from the observation that increased infiltration of macrophages into the gut mucosa was shown to be associated with depleted circulating monocytes in HIV-1 infected individuals [126]. In addition, increased dendritic cell homing to lymphatic tissues was also suggested as an explanation, at least in part, for the decreased dendritic cell numbers in the circulation of HIV-1 patients (reviewed in ref [21]).
- Therapeutic drugs. Some, but not all, antiretroviral drug classes and other drugs that are used to treat co-infections, opportunistic infections, and/or HIV-1-related or unrelated malignancies can also cause neutropenia [72, 76, 127–135]. For antiretroviral drugs, it is well-known that certain antiretroviral drugs such as Zidovudine (AZT), which is a nucleoside analog reverse-transcriptase inhibitor, have bone marrow toxicity and myelosuppression properties [132, 133]. Importantly, studies have shown that HIV-1 patients receiving AZT-containing highly active antiretroviral therapy (HAART)

are more likely to experience neutropenia [134]. Other antiretroviral drugs, particularly protease inhibitors, are also known to cause neutropenia [135]. Therefore, during the treatment of neutropenia in ART-treated HIV-1 patients, health care providers should consider the bone marrow associated myelosuppression as side effects of these drugs.

- Miscellaneous factors. Other factors such as age, ethnicity, genetics, and advanced disease stage may also contribute to neutropenia during the course of HIV-1 infection [78, 136, 137].

Taken together, these data indicate that neutropenia among HIV-1 patients is multifactorial (Fig. 1). Several clinical and experimental investigations have indicated that using cytokines that act as hematopoietic growth factors such as G-CSF and/or GM-CSF may significantly increase PMN counts by overcoming the myelosuppression observed in HIV-1 infection [70, 138]. For G-CSF, several studies have demonstrated that the application of filgrastim, a recombinant human methionyl G-CSF, to HIV-1 patients can significantly alleviate neutropenia [137, 139–141]. For GM-CSF, a clinical study has shown that treatment of leukopenic HIV-1 patients with recombinant human GM-CSF significantly increased the total leukocyte count, including PMNs [142]. Another clinical study (a phase III trial) has also demonstrated that administration of GM-CSF to HIV-1 patients at an advanced disease stage significantly increased PMN and $CD4^+$ T cell counts [143]. These hematopoietic growth factors can increase PMN generation at the level of bone marrow hematopoiesis and alleviate apoptosis in the peripheral circulation. Further investigations have shown that using other cytokines such as IL-15 can also significantly reduce the rate of PMN apoptosis [144]. Of note, clinical application of IL-15 should not be considered, because increased plasma levels of IL-15 is associated with HIV-1 disease progression [145].

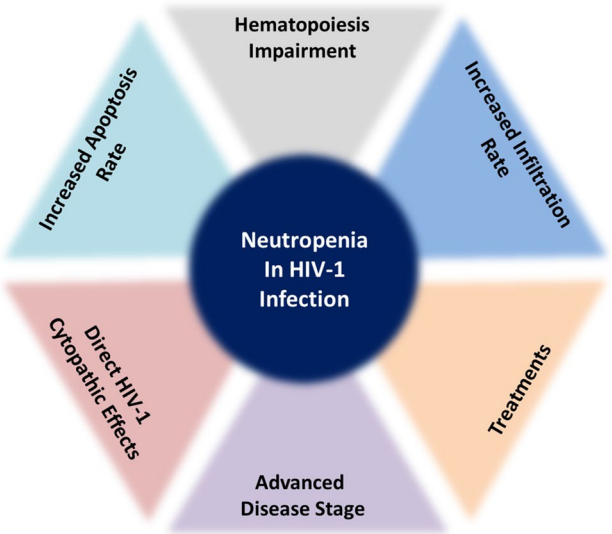

Fig. 1 Factors that drive neutropenia in HIV-1 patients

PMNs: functional defects in HIV-1 infection

It is well-established that PMNs from HIV-1 patients exhibit multiple functional defects [23, 71, 146–150]. For example, several studies have reported an impaired anti-microbial killing activity for PMNs from HIV-1 patients, especially from patients in advanced stages of disease. For instance, the capacity of PMNs from HIV-1 patients to phagocytize bacteria (e.g. *Escherichia coli* and *Staphylococcus aureus*) was significantly reduced in patients with low $CD4^+$ T cells count when compared to patients with higher $CD4^+$ T cells count and healthy individuals [151–153]. Similarly, investigators have also reported defects in the anti-fungal (e.g. *Aspergillus fumigatus* and *Cryptococcus neoformans*) activity of PMNs obtained from HIV-1 patients [154, 155]. HIV-1 and its accessory proteins such as Tat have been implicated directly in the impairment of phagocytosis and respiratory burst in different phagocytes, including PMNs [149, 150]. Of note, the level of impairment of the phagocytic activity and respiratory burst of PMNs during the course of HIV-1 infection has shown to be directly associated with the viral load and indirectly with $CD4^+$ T cell counts. PMNs from patients successfully treated with highly active antiretroviral therapy (HAART) were shown to have better functions than patients suffering from HAART failure [150], indicating that such defects are associated with faster disease progression. Other studies have reported defects in PMN development, cell structure, adhesion, chemotaxis and recruitment during HIV-1 infection [147, 148, 156, 157]. Still others reported dysregulated cytokine production and defects in degranulation in PMNs from HIV-1 patients [158–160]. Furthermore, PMNs from HIV-1 patients exhibit dysregulated responses to endotoxin stimulation and a reduced inhibitory response to S100A8 and S100A9, calcium-binding proteins that are abundant in the cytosolic compartment of human PMNs which can inhibit oxidative metabolism. This is likely to be associated with an increased risk of oxidative stress-related illnesses such as cardiovascular diseases [161]. HIV-1 can also indirectly impair PMN functions, for example, HIV-1 down-regulates NET-mediated effector functions by inhibiting their formation, through suppressing the production of ROS via IL-10 produced by dendritic cells following HIV-1 binding to DC-SIGN [23]. One should also realise that NET formation by PMNs is initiated after viral stimulation of PMNs' TLR-7 and TLR-8 receptors [23]. Furthermore, HIV-1 can impair antibody-dependent cellular

cytotoxicity, mediated by phagocytes including PMNs, partially, by enhancing the shedding of and/or down-regulating the expression level of CD16 on PMNs [162, 163]. Indeed these functional defects can negatively affect a wide range of immune responses against HIV-1, thereby contributing to the pathogenesis of HIV-1 infections.

Functional defects in PMNs from HIV-1 patients may, in part, result from the direct binding of HIV-1 or HIV-1 proteins to the cell membrane of PMN as declared by Muñoz and coworkers [164], or indirectly by altering the plasma cytokine network and cellular components of the immune system, or even due to the presence of intrinsic defects in the PMNs themselves during development [165]. Importantly, application of G-CSF and GM-CSF has been shown to abrogate PMN functional defects in HIV-1 patients [138, 139].

PMNs and the pathogenesis of HIV-1 infection

Resting PMNs, even those negative for CD4, were shown to bind to HIV-1 and efficiently enhance viral transfer to $CD4^+$ T cells in a manner dependent on cell-to-cell contact [166]. Interestingly, studies have revealed that activation of PMNs can increase the binding of HIV-1 at least twofold. These events were shown to be associated with increased transfer of HIV-1 to $CD4^+$ T cells, when compared to HIV-1 bound to resting PMNs and to free HIV-1 particles [167]. In addition, PMNs were demonstrated to significantly increase C-C chemokine receptor type 5 (CCR5 or R5)/macrophage tropic HIV-1 replication when cultured with monocyte-derived macrophages through the production of IL-10 and CCL2 in a manner independent on direct cell-to-cell contact [168, 169]. At mucosal tissues of the genital tract and in draining lymph nodes, the attachment of HIV-1 to PMNs may support the establishment of new infection by facilitating trans-infection of $CD4^+$ T cells. This, in part, could be achieved by the aid of peripheral blood mononeuclear cells, since they produce GM-CSF that prolongs PMN survival, which in turn may facilitate the interaction of HIV-1-bound PMNs with $CD4^+$ T cells [170]. Consistently, increased infiltration of PMNs to the penile foreskin in the presence of $CD4^+$ T cells has been shown to be correlated with an increased risk of HIV-1 infection [171].

PMNs can also contribute to the chronic immune activation observed during the course of HIV-1 infection, which is a hallmark of pathogenesis in HIV-1 disease progression [172], through the increased production of α-defensins in the circulation of HIV-1 patients [173]. Although high levels α-defensins could play a beneficial role in protection against HIV-1 acquisition in highly exposed but uninfected individuals [80], they may have detrimental effects in the course of natural HIV-1 infection [173]. Moreover, PMNs have been shown to express high levels of programmed death-1 ligand (PD-L1) on their surfaces [146]. Importantly, the elevated expression level of PD-L1 on PMNs has been shown to be associated with: (i) increased PD-1 expression on both $CD4^+$ and $CD8^+$ T lymphocytes; (ii) increased levels of PMN degranulation markers; and (iii) an increased frequency of PMNs expressing the granulocytic myeloid-derived suppressor cell phenotype [146]. Of note, PD-L1 interaction with PD-1 on T cells has been implicated in immune exhaustion, lower $CD4^+$ T cell counts and faster disease progression, all of which are critical in the pathogenesis of both HIV-1 and SIV infections [146, 174–177]. This may be because the interaction of PD-1 on T cells with its ligand (PD-L1) expressed on several types of immune cells, such as monocytes/macrophages and dendritic cells, negatively affects T cell function through down-regulating cytokine production and proliferation. Similarly, increased PD-L1 expression on PMNs in HIV-1 infection has been shown to suppress T cell immune responses, through the PD-L1/PD-1 pathway and ROS production [146]. As such, PD-1 blockade is suggested as a strategy to abrogate PD-1/PD-L1 mediated immune activation, exhaustion, and impairment [174–177].

Chronic immune activation is strongly believed to be associated with the development of non-HIV/AIDS-related inflammatory conditions in HIV-1 patients, even in those with well-controlled viremia (HIV-1 elite controllers or treatment responders). Furthermore, immune activation may be the underlying cause of continuous loss of $CD4^+$ T cells, especially in those with undetectable viremia [178]. To assess the role of PMNs in this context, Campillo-Gimenez and coworkers enrolled two groups of ART-treated HIV-1 patients, with and without inflammatory disorders, as well as a group of healthy individuals as a control group. Importantly, they showed that hyperactivation of PMNs was greater in those patients with inflammatory conditions [179]. Hyperactivation of PMNs was also shown to be associated with imbalanced PMNs apoptosis/necrosis [179], which, in part, could be related to impaired macrophages failing to phagocytize apoptotic PMNs in HIV-1 patients [180], thereby contributing to the chronic inflammation in HIV-1 infection. One useful marker for PMN activation and systemic inflammation during HIV-1 infection is the increased expression of CD64 (FcγRI) on PMNs, as has been recently revealed [181, 182]. Activation of PMNs during HIV-1 infection can be mediated by direct HIV-1 contact with TLRs expressed on PMNs [183]. The interaction of PMNs' TLRs with HIV-1 or HIV-1-derived single-stranded RNA has been shown to induce the production of inflammatory cytokines (such as TNF-α and IL-6) and ROS [183, 184]. Furthermore, HIV-1 Nef can also activate PMNs and induce the production of ROS [185]. Importantly, it has been revealed that there is a relationship between ROS production and TLRs [186, 187]. Consistently, increased PMN ROS production following HIV-1 interaction with TLR7/8 has

also been revealed [188], indicating that HIV-1 enhances the production of ROS, in part, through TLRs. This increased inflammatory cytokine production and ROS generation could have detrimental impacts on the site of stimulution leading to tissue damage such as epithelial barrier damage resulting in microbial translocation (discussed below), which is a critical contributor to the chronic immune activation during HIV-1 infection. These data indicate that the activation of PMNs is a consequence of HIV-1 infection and that activated PMNs, in turn, contribute to the chronic immune activation during the course of HIV-1 infection. Hence, using antioxidants and/or anti-inflammatory agents could provide a potential therapeutic strategy for HIV-1 infection, at least in part, through containing chronic immune activation.

The role of PMNs in maintaining gut epithelial barrier integrity in homeostasis and HIV-1 infection

PMNs were shown to critically impact mucosal homeostasis, especially within the gut compartment [189, "reviewed in 190, 191", 192]. This is because they play a major role in controlling microbes translocated across impaired gut epithelial barriers. This is based on their exceptional capacity to eliminate extracellular pathogens, as aforementioned, as well as their ability to participate in the healing of damaged tissues (discussed below). However; PMNs may have detrimental effects on inflamed parts of the gut mucosa, especially during certain chronic inflammatory conditions. Hence, one can conclude that PMNs may act as a double-edged sword (reviewed in [190]).

The importance of maintaining gut epithelial barrier integrity in human health

In normal adult humans, approximately, a 400 m^2 monolayer of columnar epithelial cells covers the gastrointestinal tract, also known as the gut epithelial barrier, representing the largest environmentally-exposed part of the body. Maintaining an intact gut epithelial barrier is essential for maintaining gut homeostasis (reviewed in [193–196]). This is because the gut epithelial barrier acts as a physical barrier to prevent the non-physiological passage or translocation of the gut's lumenal content (i.e. commensal microorganisms and their byproducts, and other noxious substances) to the lamina propria, deeper tissues, and/or into the blood circulation. Indeed, microbial translocation is a consequence and/or a cause of different pathological conditions such as: inflammatory bowel diseases, celiac disease, obesity, diabetes, and certain cancers (e.g. colorectal cancer) [197–204]. Furthermore, the intact gut epithelial barrier is essential for orchestrating the immune responses within the gut compartment [193–196]. These data underscore the extreme importance of maintaining both the functional and physical integrity of this barrier.

The continuous exposure of this barrier to noxious substances present in the gut lumen can compromise its integrity as time passes. To avoid this, this barrier is entirely regenerated, every three to five days on average in humans, through a strictly balanced process of senescent epithelial cells shedding at the intestinal villi and differentiation of new epithelial cells from the intestinal stem cells that reside within the intestinal crypt [205–208]. Of note, gut epithelial cells are held together by tight junctions. These junctions are composed of multi-protein complexes that form a selective permeable barrier between adherent cells [209]. To further support the integrity of this layer, a massive number of immune cells are localized within gut mucosal tissues; ready to 'accommodate' any invasion that could impair the integrity of the gut epithelial barrier [210].

The role of PMNs in controlling microbial translocation under normal conditions

During gut epithelial barrier regeneration, some of the gut lumen contents translocate across this layer into the lamina propria. Once this occurs, phagocytes (especially PMNs and macrophages) and other immune cells are rapidly recruited. These phagocytes, particularly PMNs, will contribute to the clearance of translocated microbes and prevent their dissemination into the lamina propria or deeper to the draining lymph nodes. Most importantly they prevent them from reaching the blood circulation. In fact, evidence for the very critical role of PMNs in controlling intestinal microbial translocation arose from early investigations which indicated that 50% of the infections in neutropenic cancer patients result from intestinal microbiota [211]. More recent studies have also indicated that chemotherapy-induced neutropenia in mice models is also associated with increased intestinal microbial translocation [212–215]. However, under normal conditions, recruited PMNs participate in the healing of injured intestinal epithelial barriers to prevent further microbial translocation. Nonetheless, prolonged immune activation and continuous immune cell recruitment to the site of infection/inflammation can negatively affect these tissues, which in turn can lead to certain pathological conditions (discussed below), depending on the site of inflammation. Hence, upon clearance of translocated microbes, PMNs release several agents such as lipid mediators including resolvins, protectins, and lipoxins to counteract the recruitment of other phagocytes, including PMNs. Moreover,

these lipid mediators participate in the healing of damaged epithelial barriers (reviewed in [216]). At the same time, PMNs release some proteases that degrade cytokines and chemokines at the site of cleared microbes to limit the recruitment of additional phagocytes, including PMNs, to in effect terminate inflammation. Of note, these data could provide an explanation for why the gut of healthy individuals contains a small number of PMNs, when compared to other innate effector cells [42].

Indeed, other types of immune cells are now well-appreciated to play a central role in maintaining mucosal tissue homeostasis, particularly within the gut compartments, such as the helper T cells type 17 (TH_{17}). These cells secrete different cytokines (such as IL-17 and IL-22) and chemokines that are involved in maintaining the integrity of gut compartments [217, 218]. These cytokines can act as chemo-attractants to PMNs, and play a crucial role in antimicrobial production such as β–defensin and S100, a calcium-binding protein that participates in immune defense against bacterial pathogens [218]. In addition, they participate in the healing of injured intestinal epithelial barriers by inducing the proliferation, differentiation, and tight-junction formation of epithelial cells [218, 219]. Interestingly, under normal conditions, PMNs could be involved in maintaining normal TH_{17} cells counts, since studies on mice have demonstrated that PMNs can instruct the polarization of naive helper T cells to differentiate into TH_{17} cells [220]. Taken together, these data highlight the indispensable role of PMNs in maintaining gut homeostasis under physiological conditions.

PMNs and the pathology of certain intestinal inflammatory conditions

Under abnormal conditions, PMNs can participate in the pathogenesis of certain diseases. For instance, inflammatory bowel diseases are characterized by chronic inflammation of the intestinal tract (reviewed in [221, 222]), microbial translocation [223, 224], abnormal function of PMNs [225, 226], as well as increased infiltration and activation of PMNs and other phagocytes to the gut compartments [190, 227], all of which contribute to the pathogenesis of inflammatory bowel diseases. This is because PMN infiltration to the site of infection/inflammation is associated with increased pro-inflammatory cytokine secretion (such as IL-1β, IL-6, and TNF-α), ROS generation, and MPO releasing, which is the major constituent of the PMNs' primary granules [156, 228–232]. These products, when increased at the gut mucosa, are associated with the severity of inflammatory bowel diseases [230, 233, 234], because they not only impact translocated microbes/invaders, if present, but also negatively impact the tissues at the sites of stimulation. For instance, the interaction of MPO with mannose receptors on residual macrophages leads to pro-inflammatory cytokine and ROS release by macrophages (reviewed in [53]). In turn, pro-inflammatory cytokines then increase the permeability of the gut epithelial barrier by manipulating the expression of genes responsible for tight junction formation, or through biochemical or morphological reorganization of tight junction proteins, as illustrated in (Fig. 2) [230–232, 235–237]. Equally, excessive ROS production within these

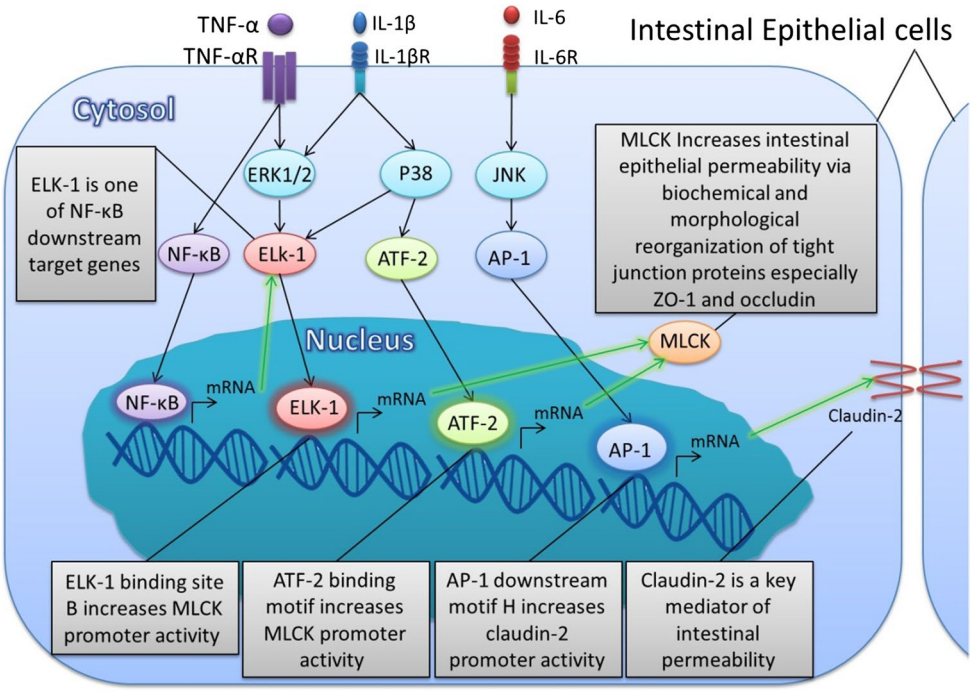

Fig. 2 Signaling pathways mediated by pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) in intestinal epithelial cells that lead to increased intestinal epithelial permeability. These signaling pathways were obtained from these references [230–232, 235–237, 271–274]

compartments participates in the induction of a high rate of epithelial cells shedding/apoptosis, thereby dysregulating the balance of apoptotic intestinal epithelial cells shedding at the intestinal villi and the differentiation of new epithelial cells from the intestinal stem cells at the intestinal crypt. In other words, the increased apoptosis/shedding rate of intestinal epithelial cells exceeds the capacity of intestinal epithelial stem cell differentiation to compensate for this high rate of cell death, manifested by crypt hypertrophy [238–240]. Indeed, not only do the PMNs' secreted molecules impact on gut epithelial barrier function, but also the PMNs themselves can significantly affect epithelial barrier function and homeostasis, since PMN transepithelial infiltration can modulate the expression, conformation, and distribution of adhesion molecules; for more details see [241]. Accordingly, increased PMN infiltration rates into the gut compartment, as a result of persistent gut inflammation and/or microbial translocation, has been demonstrated to play a central role in the impairment of gut epithelial barrier.

HIV-1 infection, gut epithelial barrier integrity, microbial translocation, and a possible role for PMNs

In the case of HIV-1 infection, there is more to say about microbial translocation, immune activation and disease progression. This is because it is well-recognized that HIV-1 mainly resides and replicates in the gut compartment (gut associate lymphatic tissues, GALT), where a huge number of immune target cells are present [242–244]. After HIV-1 infection and before microbial translocation (pre-microbial translocation stage), HIV-1 induces local inflammation in GALT, which, in part, could be mediated by pyroptosis, a form of inflammatory programmed cell death pathway induced by caspase-1, caspase-4, or caspase-5 in humans in response to different stimuli, which is associated with increased IL-1β and IL-18 production. This assumption arose from recent investigations that have shown that the intestinal paneth cells of *Rhesus macaques*, which are located at the intestinal epithelial crypt, produce IL-1β in response to SIV [232], inducing local inflammation. As a consequence of both viral replication and inflammation of GALT, the integrity of the gut epithelial barrier becomes partially impaired resulting in microbial translocation. From this point, HIV-1 infection actually shifts into another stage, specifically the post-microbial translocation stage. As a result, activation and death pathways are changed, as demonstrated by Steele and coworkers, who revealed that microbial exposure alters HIV-1-induced pyroptosis (i.e. through the caspase-1 pathway) in bystander $CD4^+$ T cells in the gut mucosa towards apoptosis (i.e. a caspase-3 pathway) in infected $CD4^+$ T cells, as a result of increasingly productive infected T cells [245]. Similarly, it has also been revealed that the exposure to *Lactobacillus plantarum* can reverse the damage mediated by IL-1β [232]. These are consistent with the recent observations that microbial translocation increases HIV-1 replication in $CD4^+$ T cells [246]. Indeed, the continuous HIV-1 replication in GALT results in (i) a massive depletion of immune cells mainly $CD4^+$ T cells, including TH_{17} and TH_{22} cells, and (ii) a great alteration in the immune components, as demonstrated both in HIV-1-infected humans and SIV-infected non-human primates [219, 238, 243, 244, 247–250]. This, in turn, alters the anatomical structure and functional activities of these compartments leading to impaired GALT integrity [238, 250–257], supporting additional microbial translocation [238, 249].

It is important to realise that although the alteration and depletion of immune cells in GALT contributes to the impairment of gut epithelial barrier integrity, HIV-1 and its proteins (such as Gp120) can also do this, leading to microbial translocation [254]. Two decades ago, Delézay and coworkers demonstrated that exposure of HIV-1 to HT-29-D4, a human colonic epithelial cell line, can impair their differentiation, in part, by affecting epithelial barrier function [255]. Of note, some *in vitro* studies have also indicated that HIV-1 could directly infect epithelial cells [256–259]. Interestingly, according to these studies, HIV-1 infection of epithelial cells could be associated with inflammatory cytokine secretion [258, 259]. Other studies have shown that epithelial cells naturally resist HIV-1 infection but instead have demonstrated that HIV-1 can bind to and interact with epithelial cells [254, 260, 261]. For instance, Nazli and coworkers have demonstrated that the exposure of T84, an intestinal cell line, to HIV-1 or its gp120, but not Tat protein, increased their permeability as a result of disruption to the tight junctions [254]. These events were also shown to be associated with increased inflammatory cytokine secretion, including TNF-α [254]. Furthermore, HIV-1 replication in GALT has been implicated in Wnt and TGF-β signaling pathway dysregulation, pathways which are involved in intestinal epithelial cell migration and differentiation [238]. This could explain why 'well-controlled' HIV-1 replication in GALT was shown to associate with better gut epithelial barrier function [238], and thus less microbial translocation. This process is actually observed in HIV-1 elite controllers (who do not progress to AIDS naturally and have considerably lower levels of microbial translocation and immune activation), reflecting the critical impact of HIV-1 replication and gut epithelial barrier integrity in the pathogenesis of HIV-1 infection in humans [262, 263]. Similarly, the absence of microbial translocation and immune activation in chronically SIV-infected Soot mangabeys (monkeys that do not progress to AIDS naturally) provides evidence for the critical correlation between viral replication and gut epithelial barrier integrity and disease progression [263, 264].

On the other hand, a study was conducted to assess the function of the intestinal epithelial barrier using HT-29/B6, a colonic epithelial cell line, upon exposure to HIV-1-infected immune cells [265]. In this study, Stockmann and colleagues have shown that HIV-1 infected immune cells can impair the function of the intestinal epithelial barrier, at least in part, by secreting pro-inflammatory cytokines [265]. Similarly, the continuous infiltration of immune cells, particularly phagocytes (such as PMNs and macrophages), to the site of inflammation can worsen the inflammatory status of these tissues, at least in part, by increasing pro-inflammatory cytokine secretion (Fig. 3). This adds another mechanism that can explain the pathogenesis of GALT damage and increased microbial translocation in HIV-1 infection.

To further support the role of increased PMN infiltration into an organ (tissues) in the pathology of that organ (tissues), Puerta-Arias et al. [266] showed that PMNs themselves can contribute to the pathogenesis of fibrosis and pulmonary inflammation in mice, in which increased PMN infiltration was observed, through secreting pro-inflammatory cytokines. As such, depletion of PMNs was proposed as a strategy to promote the resolution of fibrosis and pulmonary inflammation in mice, in part, by down-regulating the production of pro-inflammatory cytokines [265]. Consistently, the continuous infiltration of PMNs has also been

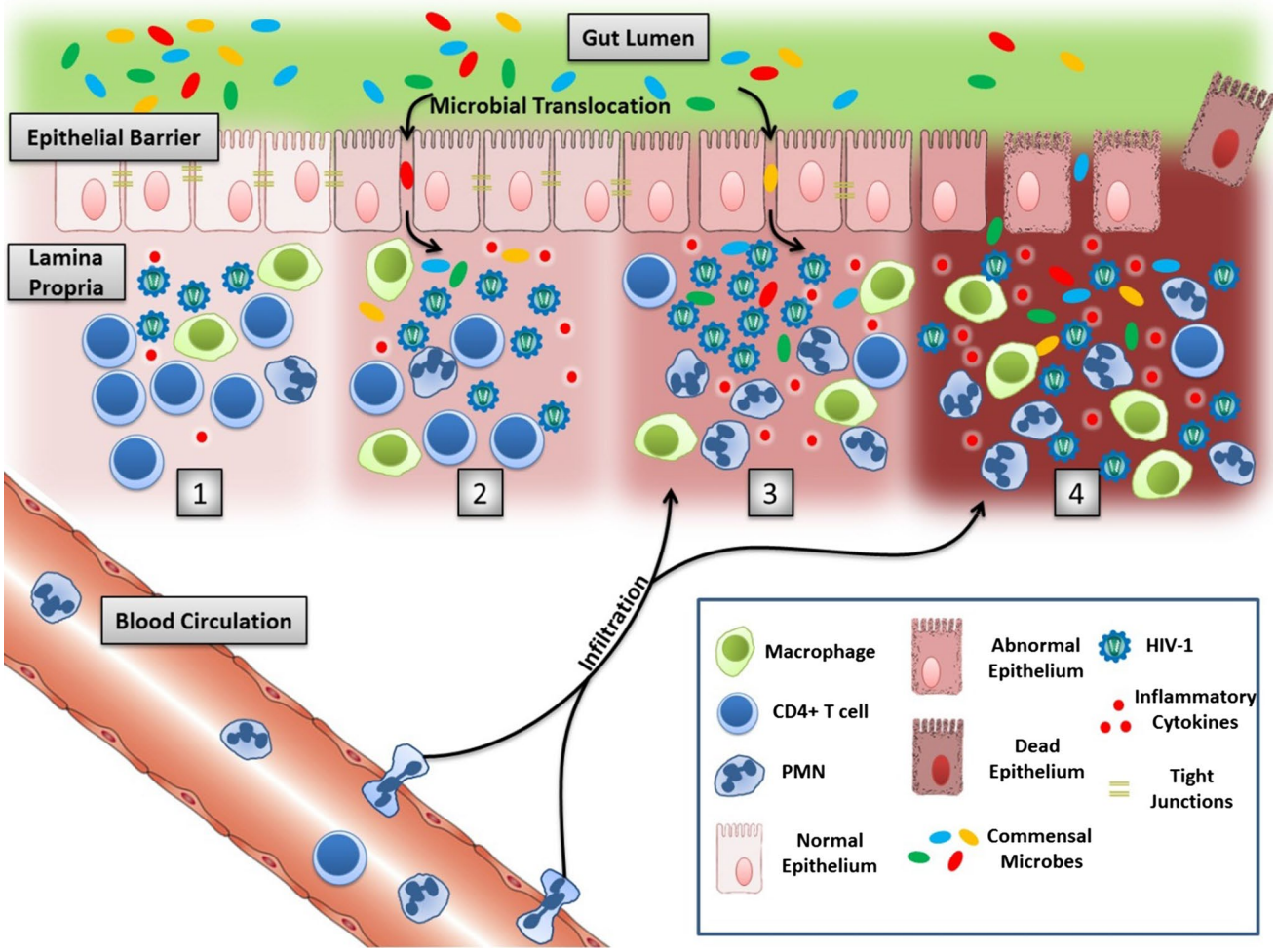

Fig. 3 A proposed model describing the role of PMNs in the impairment of gut epithelial barrier integrity and microbial translocation during HIV-1 infection. After HIV-1 infection (sexual or non-sexual in nature) HIV-1 disseminates to the gut associated lymphatic tissues where a pool of immune cells is present, as seen in case number 1. Exposure of the gut epithelial barrier to HIV-1 particles can impair barrier integrity. Furthermore, HIV-1 replication, depletion of immune cells (particularly $CD4^+$ T cells) and increased inflammatory cytokine production can also contribute to epithelial barrier integrity damage (such as decreased tight-junctions expression), resulting in microbial translocation as seen in case number 2. Microbial translocation increases immune activation, inflammation and HIV-1 replication supporting additional microbial translocation and triggering the infiltration of phagocytes such as PMNs and macrophages, case number 3. Unfortunately, as in case number 4 the increased infiltration of PMNs and macrophages within the gut mucosa, can only worsen the inflammatory condition of these tissues leading to permanent damage to the gut epithelial barrier

implicated in lung-pathology in HIV-1-infected humanized mice co- or not infected with *Mycobacterium tuberculosis* [266]. Interestingly, in both cases, there was a remarkable increase in pro-inflammatory cytokine (IL-1β, IL-6, TNF-α, and IL-8) secretion [267]. On the other hand, the increased infiltration rate of phagocytes (i.e., macrophages) to the gut mucosa has been reported in HIV-1 patients, which was also shown to be associated with increased pro-inflammatory molecules related to macrophages within these compartments [126]. As such, the accumulation of macrophages in the gut mucosa has been suggested as a contributor in the pathogenesis of HIV-1 infection through promotion of inflammation [126]. Interestingly, macrophages that accumulate in the colon of AIDS patients were demonstrated to be responsive to LPS and express inflammatory cytokines such as IL-1β and TNF-α, supporting the role of macrophages in the pathogenesis of the gut mucosa in HIV-1 infected individuals [268]. Similarly to macrophages, increased PMN infiltration to the gut compartments of chronically SIV-infected *Rhesus macaques* has also been demonstrated [264]. Importantly, this study showed that the lamina propria of SIV-infected *Rhesus macaques* contains an increased level of MPO^+ PMNs; this observation was shown to be associated with epithelial barrier damage and increased microbial translocation [264]. These infiltrated PMNs could participate in the pathology of gut mucosa by increasing the secretion of inflammatory cytokines (Fig. 2 and Fig. 3), MPO, and by generating ROS. Moreover, Somsouk and coworkers have demonstrated that HIV-1 infected individuals, even those treated with ART, have significantly high rates of PMN infiltration into gut mucosal tissues, and this event was shown to be associated with increased mucosal apoptosis [269], both of which can also contribute to microbial translocation (Fig. 3). Microbial translocation contributes to the pathogenesis of HIV-1 infection by driving chronic immune activation, which is now recognized as a critical predictive marker for faster disease progression in HIV-1 and SIV infections. Indeed, certain translocated microbes, namely Gram negative bacteria, can enhance viral replication by increasing the expression of the CCR5 receptor on $CD4^+$ T cells present in the lamina propria [246], amplifying this vicious cycle. It should be noted that HIV-1 is among those pathogens that thrive under highly inflammatory conditions (high pro-inflammatory cytokines and ROS) [270].

Taken together, these data indicate that impairment of gut mucosal integrity and increased microbial translocation in HIV-1 infection is, at least in part, a result of increased inflammatory conditions (inflammatory cytokines and ROS) mediated by an increased infiltration rate of phagocytes, including PMNs, to the gut mucosa of HIV-1-infected individuals. However, additional investigation is needed to further establish the role of PMNs in gut epithelial barrier integrity and microbial translocation during the course of HIV-1 infection at different clinical stages of disease (acute, chronic, and AIDS).

Conclusion

PMNs are critical innate immune cells involved in the clearance of pathogens. They are considered the most powerful immune cells in eliminating pathogens, especially extracellular ones. Additionally, they play a vital role in regulating innate immune responses, since they cross-talk with different innate immune cells. Furthermore, PMNs can directly instruct polarization and activation of specific adaptive immune responses. These data underscore the critical role that PMNs play in pathogen elimination and immune response mediation and regulation.

In the case of HIV-1 infection, neutropenia is relatively common in HIV-1 patients. Neutropenia is known to be associated with recurrent microbial infections, particularly, during the advanced stages of HIV-1 disease. Of note, several factors can lead to neutropenia in HIV-1 patients, including increased peripheral apoptosis rates, decreased production rates at the level of hematopoiesis, increased rates of infiltration, as well as certain drug treatments. Of central importance, the neutropenia observed during HIV-1 infection is not only known associated with increased microbial infections but also contributes to defects in immune function. On the other hand, PMNs become defective as HIV-1 disease progresses, and these defects are associated with immune response impairment and increased microbial infection. In addition, PMNs from HIV-1 patients exhibit hyperactivation that can contribute to chronic immune activation and immune exhaustion, both of which are known to contribute to disease progression in HIV-1 patients. Therefore restoring normal PMN count and function is essential for preventing microbial infection and immune impairment. To this end the therapeutic application of G-CSF and GM-CSF to HIV-1 patients is suggested.

Finally, HIV-1 mainly resides and replicates in lymphatic tissues, especially, in the GALT. These tissues become chronically inflamed during the early events of HIV-1 infection. This, in turn, leads to gut integrity damage, and as a consequence, microbial translocation occurs. Both events lead to an increased phagocytic infiltration rates, particularly of PMNs. Unfortunately, once at the GALT, PMNs become fully trapped in the viral 'illusion'. These cells worsen the inflammation status of the GALT, by increasing the production of inflammatory cytokines and ROS that results in further damage to the integrity of the gut mucosa. Hence, therapeutic application of antioxidants and/or anti-inflammatory agents could provide a potential strategy for inhibiting HIV-1 infection through containment of chronic immune

activation, particularly within the GALT, which could limit phagocyte infiltration, including that of PMNs.

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Compliance with ethical standards

Conflict of interest All authors declare that this review manuscript was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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